

Dissolved Iron Stimulates Uptake of Organic Phosphorus by the Diatom *Didymosphenia*
geminata

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REU Student Research

Summer 2010

ABSTRACT

Recent blooms of *Didymosphenia geminata* in streams around the world where they had never been recorded before has generated new interest in the biology and ecology of this diatom. The species is often found in oligotrophic mountain streams where phosphorus limitation may determine productivity. Excessive stalk production that results in blooms may be a response to low phosphorus conditions. The available form of phosphorus and the concentration of iron were manipulated to determine whether these factors affect phosphorus uptake rate. Phosphorus form, iron concentration, and initial phosphorus concentration all had significant effects on uptake rate. The results of this experiment show that organic phosphorus may be an important source of phosphorus for *D. geminata*, and that high concentrations of iron may influence the phosphorus uptake mechanism. Therefore, stream chemistry is likely to play an important role in determining bloom formation by *D. geminata*.

KEYWORDS: Didymo, aquatic ecology, nutrient uptake, phosphorus, algae

INTRODUCTION

Didymosphenia geminata is a stalked diatom that recently began forming nuisance blooms in watersheds worldwide where they were never observed before. It now exhibits bloom behavior around the world. Cells of *D. geminata* secrete a stalk made of exopolysaccharides (EPS) that connects the cell to its anchoring structure on the substrate and elevates the cell into the water column. When the cells divide in vegetative reproduction, the stalk also divides and becomes branched (Spaulding and Elwell, 2007). The production of these stalks can lead to the accumulation of thick (~3 cm or more), dense mats that characterize nuisance blooms (Whitton *et al.* 2009). During blooms the algae forms dense mats that can cover 100% of the stream bottom and can extend for more than one kilometer of stream length (Spaulding and Elwell, 2007). Stalks are often more than 10 times the length of the diatom cell, and can account for more than 90% of the biomass of a mat of algae (Kirkwood *et al.*, 2007). The stalks are resistant to degradation and persist after the death of the diatom cell that secreted it (Spaulding and Elwell, 2007).

The blooms of *D. geminata* in oligotrophic streams present a paradox. The significant biomass generated in these blooms stands in contrast to the low nutrient supply, since algae blooms are generally found in nutrient-enriched waters (Spaulding and Elwell, 2007). Additionally, blooms only form in certain streams in areas where *D. geminata* is thought to be native, and cells of *D. geminata* can be found in algal samples from bloom and non-bloom streams alike. The East River watershed in Gunnison County, Colorado is an example of an area where this is the case (B.W. Taylor, unpublished data). Hydrologic, chemical, and genetic determinants of bloom behavior have all been suggested and are under investigation (Spaulding and Elwell, 2007, Miller *et al.* 2009, Whitton *et al.* 2009).

One survey of streams that support *D. geminata* blooms in Norway found that *D. geminata* was present only where total phosphorus concentrations were less than 20 µg/L, and required some organic enrichment (between 2 and 6.5 mg C/L) (Lindstrom and Skulberg, 2007). Streams with blooms in England were shown to have concentrations of dissolved organic phosphorus much higher than soluble reactive phosphorus. Importantly, stalks of *D. geminata* showed phosphomonoesterase (PMEase) activity when stained, indicating that *D. geminata* may be able to utilize organically bound phosphorus from the water column (Ellwood and Whitton, 2007). Recent experiments have shown that additions of inorganic phosphate stimulate increased cell division rates but longer stalks occurred only under lower nutrient conditions (Bothwell and Kilroy, 2010, Kilroy and Bothwell, 2010).

Studies that relate phosphorus concentration to blooms fail to explain the global increase in bloom behavior during the past 20 years, because there is no known global trend reducing phosphorus concentrations in oligotrophic stream waters. However, there are some possible relationships between global trends and phosphorus in streams. For example, increased nitrogen deposition rates over much of the northern hemisphere could intensify phosphorus limitation conditions in lakes and streams that they feed (Elser, 2009), as well as a suite of biogeochemical affects on soils and wetlands that could impact streams (Vitousek *et al.*, 1997, Bragazza, 2006). Additionally, if climate change or other factors increased the duration or extent of anaerobic conditions in beaver pond sediments, an increase in sulfate reduction by microbes could cause greater amounts of phosphorus and iron to be released from precipitates in the sediment (Klotz, 1998). This new dissolved phosphorus and iron may be released earlier than normal in a spring pulse, possibly decreasing the concentration of dissolved phosphorus available later in the growing season (B. W. Taylor, pers. comm.).

Iron may play a role in phosphorus uptake by *D. geminata*. Maurer (2008) found that dead *D. geminata* stalk material sorbed phosphate, but not after it was washed with EDTA. The EDTA wash and water rinses contained significant amounts of iron. S. Spaulding recently found that phosphorus uptake increased when iron was added to the stream water, and that streams containing *D. geminata* contained more dissolved iron than those without, by about an order of magnitude (B. W. Taylor, pers. comm.).

More information is needed on the utilization of phosphorus by *D. geminata* and other environmental variables influencing stalk production to understand the factors that cause blooms. *D. geminata* existed for years in oligotrophic streams in the Rocky Mountains and elsewhere without exhibiting bloom behavior (Whitton *et al.*, 2009, B.W. Taylor, pers. comm.). Identifying environmental factors determining the bloom behavior is important for the management of this nuisance species. We measured phosphorus uptake by periphyton to determine if *D. geminata* tuft presence, organic versus inorganic phosphate, or dissolved iron concentration influenced uptake rate relative to total algal biomass.

METHODS

Substrate was taken collected at three sites in the East River watershed: two sites were on Coal Creek near Irwin, CO, and one site was on Washington Gulch in Mt. Crested Butte, CO. Substrate taken from the same site but with and without obvious *D. geminata* tufts were placed in 1-gallon plastic bags with 2 liters of stream water spiked with phosphorus and/or iron in a two-by-two-by-two factorial design (bloom/non-bloom, inorganic phosphate/organic phosphate, iron/no iron). The rocks were exposed to the solution in the bag for two hours. The experiment duration of two hours, as well as the concentration of phosphorus additions (20 ug P/L for both inorganic and organic phosphorus) were determined to yield measurable uptake in a pilot

experiment. The amount of the iron addition, 300 ug Fe/L, was chosen since it would approximately double the ambient dissolved iron concentration, which is typically around 300 ug/L in this area (Coal Creek Watershed Coalition, 2007; Shanklin & Ryan, 2008). Water samples for phosphorus analysis were removed before the rock was added and again at the end of 120 minutes. To control for uptake or release of phosphorus other than by the rock, the experiment was performed in identical plastic bags without any added rock.

Phosphorus analysis was performed using the ascorbic acid method and acid persulfate digestion (for bags that received organic phosphorus additions only) (APHA, 1998). To measure the algae added to each bag, rocks were scrubbed using wire brushes. The material removed from the rock surface was dried at approximately 60 °C, weighed, and ashed in a muffle furnace set to 450 °C. The mass after ashing was subtracted from the dry mass to give ash free dry mass (AFDM). Individual bags with their own substrate served as replicates for each treatment combination. Analysis of covariance (ANCOVA) was performed using JMP v. 8.0.2 (SAS Institute Inc. 2009) to determine the effect of treatments on uptake. A significance threshold (α) of 0.05 was used.

RESULTS

AFDM had no significant correlation with uptake rate (Figure 1), and was not included in the final model. Therefore, uptake was compared without any standardization for the size of the rock or the amount of algae taking up phosphorus. Surface area data for the rocks will allow us to standardize for rock size but was not available at this writing. Instead, the initial concentration of phosphorus in the bag had a strong effect on phosphorus uptake rate (Figure 2), and was used as the covariate in the ANCOVA model. Treatment effects included in the ANCOVA model were initial phosphorus concentration, presence or absence of *D. geminata* tufts on rocks, iron

treatment (added/not added), and phosphorus treatment (inorganic/organic), plus all possible crosses of *D. geminata* tufts, iron treatment, and phosphorus treatment.

The model was significant overall with an R^2 of 0.579 ($F=12.395$, $p<0.001$). Uptake rate increased with higher initial concentrations ($F=83.346$, $p<0.001$, Figure 2) and with the inorganic phosphorus treatment ($F=36.099$, $p<0.001$). Additionally, the 3 way cross of *D. geminata* tufts, iron treatment, and phosphorus treatment was significant ($F=6.206$, $p=0.015$). A post-hoc Tukey Honest Significant Difference test was performed to determine which treatment combinations were significantly different (Table 1, Figure 3).

DISCUSSION

This study was designed to explore the effects of stream chemistry on *D. geminata* bloom formation by examining phosphorus uptake. Rocks with *D. geminata* mat formation were expected to show greater uptake due to their greater amount of algal biomass. Instead, the effect of the presence of a *D. geminata* mat depended on the phosphorus and iron treatments, and no pair of the same iron and phosphorus treatment was significantly different because of *D. geminata* presence. The fact that neither AFDM nor *D. geminata* mat presence (which are highly correlated) had an effect on uptake suggests that the non-living stalk material, or perhaps a large portion of it, does not take up phosphorus. Instead, uptake rate may be better correlated to the actual living biomass, which may be similar between rocks with and without *D. geminata* mat formation. The future inclusion of rock surface area data may allow some standardization for the amount of algae taking up phosphorus. Given that neither the presence of *D. geminata* tufts, nor the AFDM on the rocks affected the uptake rate, total in stream uptake rate may be comparable

between blooming reaches and non-blooming reaches of streams that contain *D. geminata* blooms.

Iron was predicted to increase inorganic phosphorus uptake rate because iron might be involved in the phosphorus uptake mechanism for *D. geminata*. Instead, iron only had a significant effect on the uptake of organic phosphate by rocks with *D. geminata* tufts, significantly increasing organic phosphorus uptake at higher concentrations. In fact, a net release of organic phosphorus was observed by blooming rocks without added iron, but uptake occurred with the addition of iron to the bag. Assuming that *D. geminata* cells are present on the non-blooming rocks, but are not producing stalk material, this finding may indicate that stalk material is important for organic phosphorus uptake in the presence of iron.

Inorganic phosphorus uptake tended to be higher than organic uptake after adjusting for initial concentration, as indicated by the least squared means shown in Table 1. Uptake of organic phosphorus by rocks with *D. geminata* mats and with added iron was significantly higher than all four organic phosphorus treatment combinations. This experiment compared the uptake rates at approximately equal initial concentrations, however. Since the initial concentration of phosphorus has a strong effect on uptake rate, in stream uptake rate of organic phosphorus may be much greater than that of inorganic phosphorus, since organic phosphorus concentrations can be four to ten times greater than inorganic phosphorus concentrations in the streams studied here (B. W. Taylor, unpublished data).

D. geminata blooms may alter stream ecosystems by changing the physical structure of the stream bottom, altering the benthic macroinvertebrate community, and changing the growth rate of trout (B. W. Taylor, unpublished data). With blooms forming in new streams every year, identifying the factors determining bloom behavior is important for determining conservation

priorities and methods; understanding global changes that may be anthropogenic, if such a change is driving the bloom development; and for general invasive species biology insights.

The results of the uptake experiment reported here show that *D. geminata* does utilize organic phosphorus, which is consistent with the presence of alkaline phosphatase enzymes on the stalk material reported by Elwood and Whitton (2007). Additionally, higher dissolved iron concentration significantly increased the uptake of organic phosphorus, but only when stalk material was present. Therefore, both dissolved phosphorus fractionation and iron concentration may be important determinants of bloom behavior on a regional scale if stalk generation is a response to the severity of phosphorus limitation as has been proposed elsewhere (Whitton, 2009, Bothwell and Kilroy, 2010, Kilroy and Bothwell, 2010). These stream chemistry parameters may also be involved in the recent global increase in the number of streams containing *D. geminata* blooms, if environmental changes operating over continental or global scales are altering stream iron or phosphorus biogeochemistry.

ACKNOWLEDGEMENTS

This project was funded by the National Science Foundation REU program at the Rocky Mountain Biological Laboratory, and supported by Sterling College. I thank Dr. Brad Taylor for all his guidance and support; Karl Grunseich, Erin Larson, and Danny O'Donnell for their assistance in completing the field work; and Farley Brown, Ned Houston, and Dr. Charlotte Rosendahl for their advice and encouragement.

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FIGURES AND TABLES:

Table 1: Least Square Means and Tukey HSD Comparison Results.

Treatment Combination	Least Square Mean	Tukey HSD comparison
Bloom/Inorganic P/With Iron	0.174	A
Bloom/Inorganic P/No Iron	0.169	AB
Non-Bloom/Inorganic P/With Iron	0.151	AB
Non-Bloom/Inorganic P/No Iron	0.108	ABC
Bloom/Organic P/With Iron	0.019	BCD
Non-Bloom/Organic P/No Iron	0.001	CDE
Non-Bloom/Organic P/With Iron	-0.035	DE
Bloom/Organic P/No Iron	-0.115	E

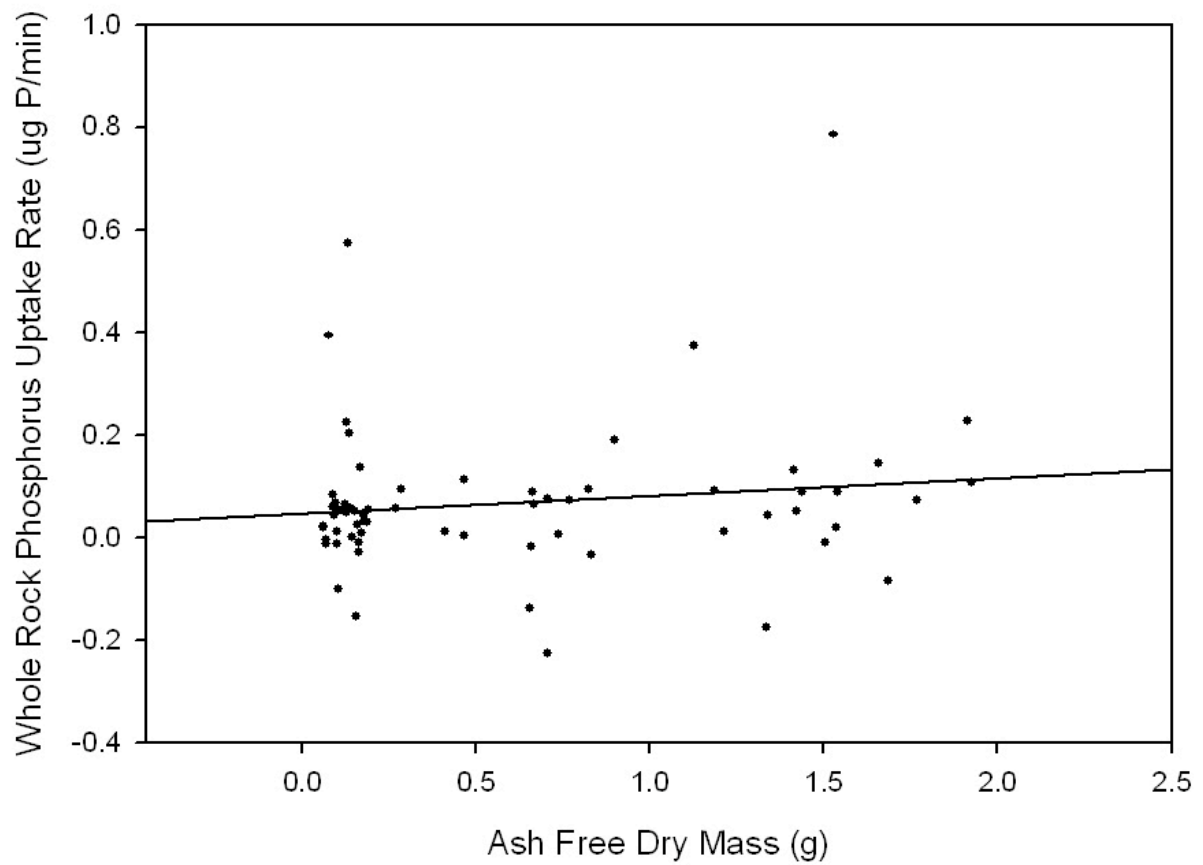


Figure 1: The regression of whole-rock uptake rate (the total mass of phosphorus removed from the bag divided by the duration of the experiment) and ash free dry mass (AFDM). There is no significant relationship between whole rock uptake rate and AFDM ($R^2=0.018$, $p=0.274$).

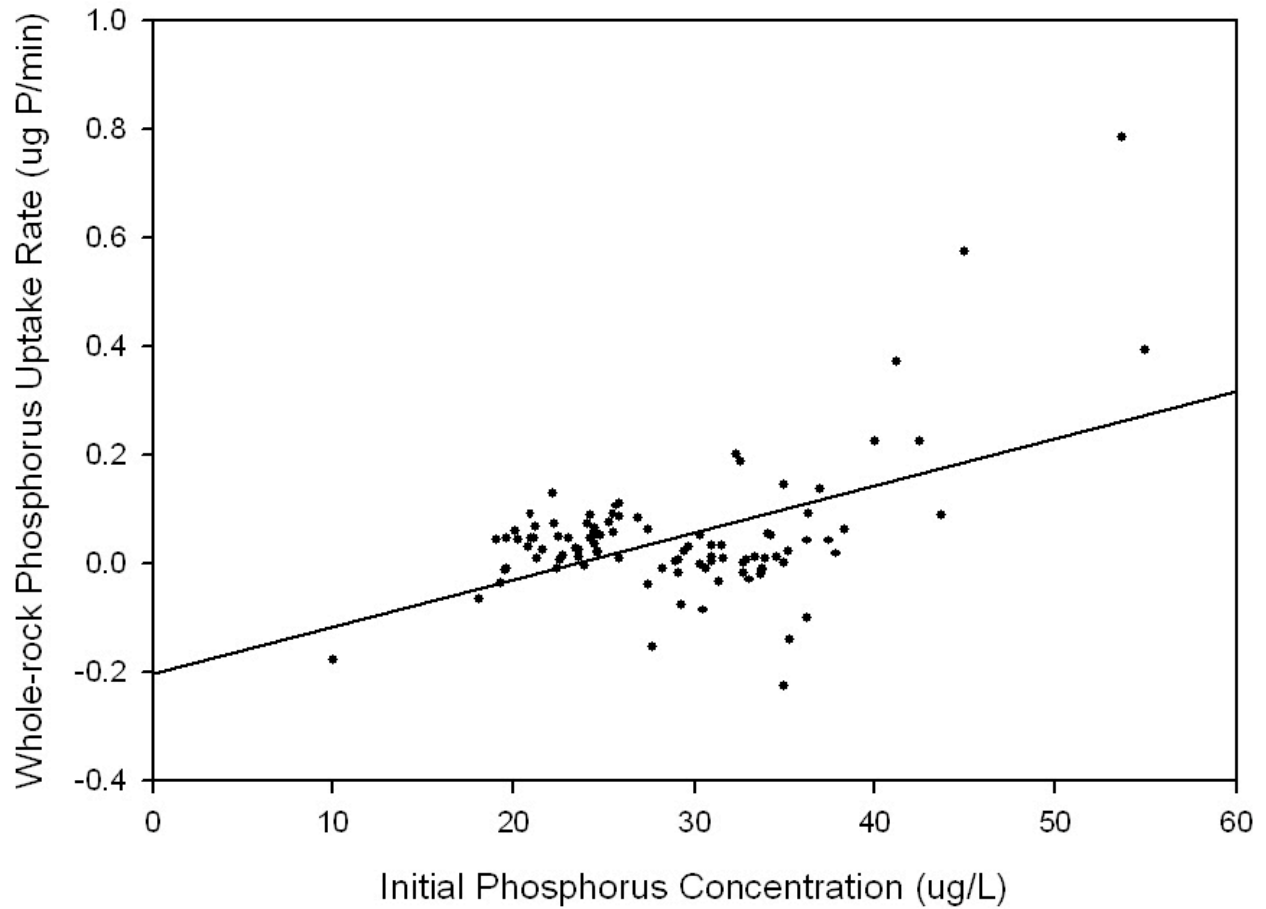


Figure 2: The regression of whole-rock uptake rate (the total mass of phosphorus removed from the bag divided by the duration of the experiment) with the measured initial phosphorus concentration prior to adding the rock. Whole rock uptake rate is significantly correlated with initial phosphorus concentration ($R^2=0.282$, $p<0.001$), and initial phosphorus concentration was a significant effect in the ANCOVA.

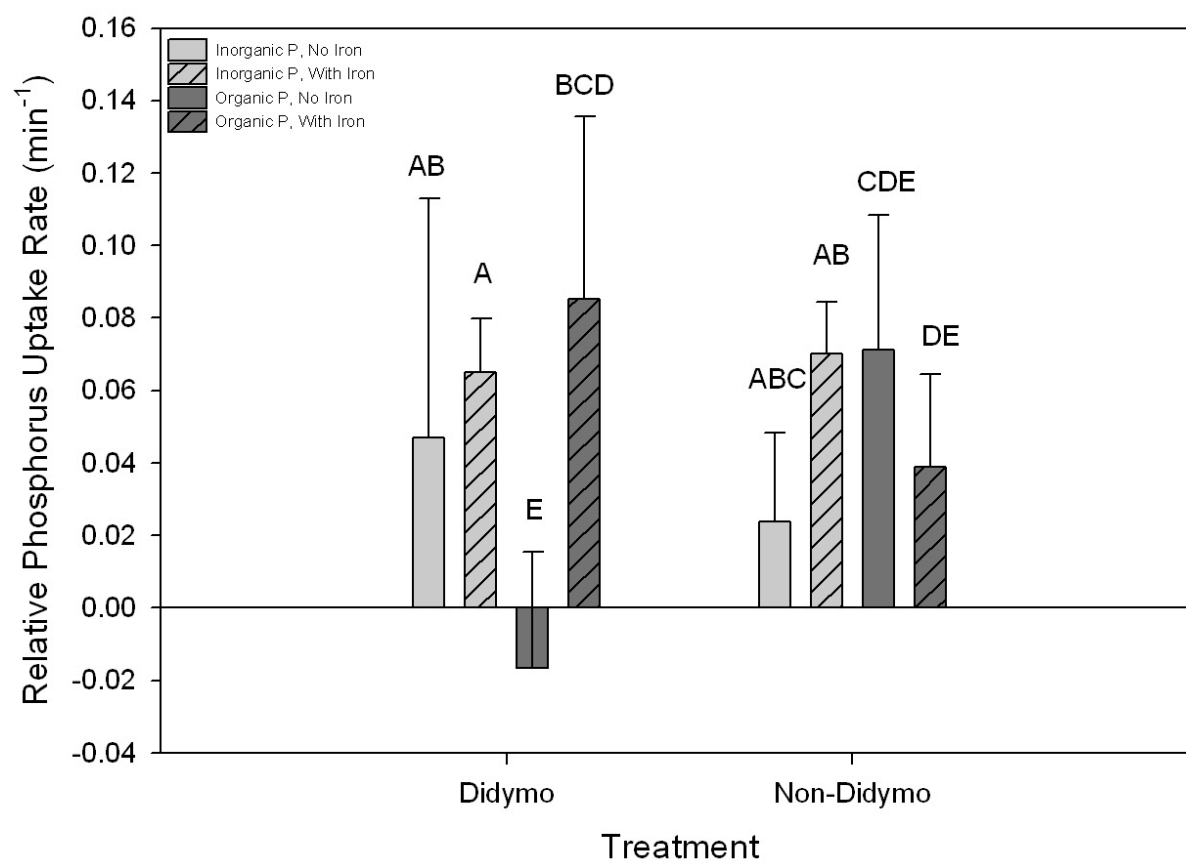


Figure 3: Mean relative uptake rate (equal to whole rock uptake rate divided by the initial mass of phosphorus in the bag) $\pm$ 1SE, by treatment combination. The results of the Tukey HSD on the least squares mean for the treatment are shown above the bars. Bars not linked by a common letter are significantly different from one another.